

## **Original Research Article**

### **Preclinical Evaluation of Anxiolytic Activity of *Aristolochia Indica* (Linn) Leaf Extract in Swiss Albino Mice**

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**Abstract:** The light/dark test is based on the aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light. The test apparatus consists of a small dark safe compartment (one third) and a large illuminated aversive compartment (two thirds). The light/dark test may be useful to predict anxiolytic-like or anxiogenic-like activity in mice. The aim of current study was to evaluate Methanolic extract of *Aristolochia Indica*. L (MeAI) for its anxiolytic potential, for this purpose the light/dark box activity test used which is commonly used to assess anxiety-like behaviours and validate the pharmacological effects of neuroactive plant extracts and compounds. MeAI at 100 mg/kg i.p exhibited significant increase in time spent in light area with respect to control animals. Moreover the reduction in anxiety behaviour, also demonstrated by significant increase in number of entries in the light compartment relative to the dark compartment of the testing apparatus.

**Keywords:** Light-dark box, *Aristolochia Indica*, Anxiolytic activity, bright illumination.

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#### **INTRODUCTION**

*Aristolochia indica* (Linn) commonly called Iswarmul is a rare endangered medicinal plant of India. It is reported to be stimulant, used for diarrhoea and intermittent fever this plant is a remedy for, drops, loss of appetite and good for snake bite. *Aristolochia indica* is a shrubby or herbaceous vine with a woody root stock and the plant is used in indigenous system of medicine. Leaves of this plant are used to treat cholera, fever and bowel troubles. As a blood purifier the dried roots & rhizomes are used as a gastric stimulant & bitter tonic. The root is used in skin diseases. It heals wounds & destroys the toxic effect of all poisons. In olden days, it was used against snakebites. In traditional medicine the underground parts of the plant are rubbed with honey & given to treat leprosy & macerated with black pepper [1-3].

#### **LIGHT- DARK BOX TEST**

Many authors have used the light dark box in different dimensions for their research in different behavioural aspects. Typical dimensions of the compartment are generally one third for the dark compartment and two thirds for the light compartment. The model is based on the observation that although nocturnal rodents such as mice will naturally tend to explore a novel environment, open fields appears to have aversive properties which inhibit exploratory behaviour. The apparatus consisted of two

polyvinylchloride boxes of the same size. One was darkened with cardboard and the other was brightly illuminated; an opaque plastic tunnel separated the two compartments [4].

#### **EXPERIMENTAL ANIMALS**

All experimental protocols and procedures were approved by the Institutional Animal Ethics Committee of Chalapathi Institute of Pharmaceutical Sciences. Male Swiss albino mice, weighing 20–25 g, overnight fasted were used throughout the study. The animals were housed in standard laboratory conditions (12-h light/dark cycle,  $21 \pm 1^\circ\text{C}$ , and relative humidity of  $55 \pm 5\%$ ) with free access to food and water prior to the experiments. After 7 days of acclimatization to laboratory conditions, the animals were randomly assigned to experimental groups, each consisting of 4 rats. Each animal was used only once in the experimental procedures. All experiments were carried out between 9 a.m. and 3 p.m.

#### **MATERIALS AND METHODS**

##### **TREATMENT GROUPS:**

Group-1 - Control group (0.9% normal saline 1ml/ kg, s.c)

Group-2 - Standard group (Diazepam 2 mg/kg, i.p.)

Group-3 - Chloroform leaf extracts (AICE 100mg/kg, i.p.)

**PROCEDURE:**

Mice were divided into 3 groups. Mice in each group are weighed, marked with picric acid. Each group is kept in different mice cages. The mouse is placed for 05min (300sec) min in a box made of two compartments, one white and lit and the other dark.

Two parameters are recorded.

- The percent of time spent in the lit compartment which is an index of the anxiety-related behaviour. Anxiety is considered to be high if the percent of time spent in the lit compartment is low (i.e. < 50%).

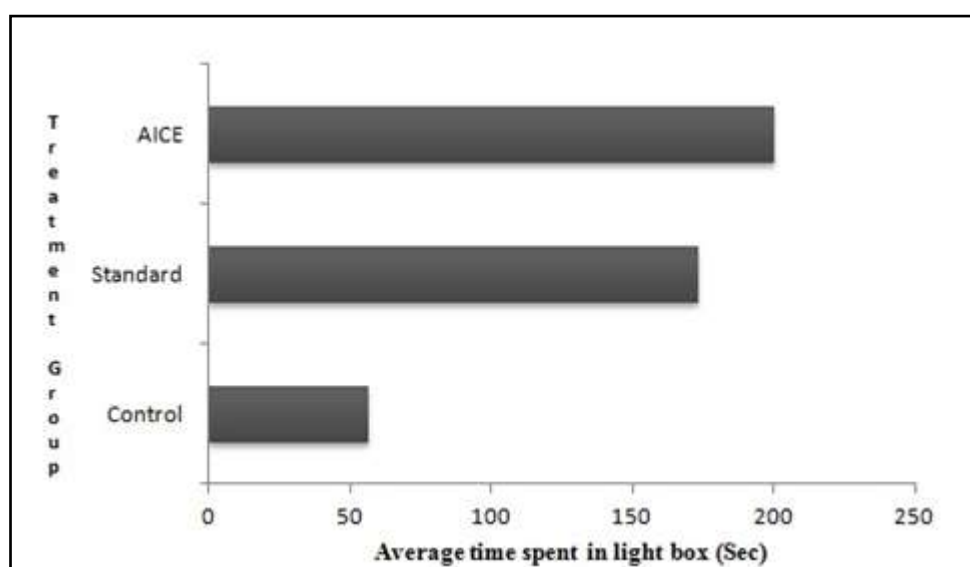
- The number of transitions between compartments. This parameter is an index of anxiety-related behaviour, but also of the motor-exploratory activity. Anxiety is considered to be high if this index is low, indicating a motor inhibition [5-13].

**RESULTS**

The chloroform leaf extract of *Aristolochia indica* has shown significant anxiolytic effect (Figure No.1 & Table No: 1).

**Table-1: Anti anxiety activity of leaf extract of *Aristolochia Indica* using the light dark box method for 5min as evaluation time.**

S. No	Treatment	Groups	Time spent in dark box (sec)				Time spent in light box (sec)				Avg. no. of light box entries	Mean $\pm$ SEM for time spent in Light Box	Mean $\pm$ SEM for entries in Light Box
			0	15	30	60	0	15	30	60			
1	Control	A	242	239	240	220	58	61	60	80	4	54.62 $\pm$ 10.57	3.5 $\pm$ 0.645
		B	264	266	258	255	36	34	42	45	5		
		C	228	224	203	227	72	76	97	73	3		
		D	274	271	263	252	26	29	37	48	2		
2	Standard	A	142	125	112	107	158	175	188	193	9	173.43 $\pm$ 2.15	7.7 $\pm$ 0.75
		B	163	147	115	102	137	153	185	198	7		
		C	134	126	120	121	166	174	180	179	6		
		D	141	132	121	117	159	168	179	183	9		
3	Chloroform extract	A	117	105	96	98	183	195	204	202	11	200.43 $\pm$ 2.72	10.5 $\pm$ 0.645
		B	112	110	94	81	188	190	206	219	9		
		C	107	91	94	76	193	209	206	224	12		
		D	115	111	96	90	185	189	204	210	10		



**Fig-1: Determining anxiolytic effect of *Aristolochia indica* by considering treatment group on Y-axis and average time spent in light box on X-axis.**

**CONCLUSION**

As with many experimental protocols, drugs that effect general motor function will affect light/dark performance. Preliminary screening of locomotor

activity such as an open field or an actimeter test appears to be necessary and sufficient for eliminating false positive result. In this current research, it was found that the leaf extract of *Aristolochia indica* has

anxiolytic activity and has to be evaluated further on much complicated procedures to identify many parameters.

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